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## STUDIES ON MEMBRANE TRANSPORT V. TRANSPORT OF LONG CHAIN FATTY ACIDS IN *HYMENOLEPIS DIMINUTA* (CESTODA)<sup>1</sup>

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Several workers have studied the incorporation of higher fatty acids into the lipids of *Hymenolepis diminuta*. Various <sup>14</sup>C-labeled fatty acids including palmitate, stearate, oleate, and linoleate were incorporated into triglycerides of *H. diminuta*, *in vitro* (Jacobsen and Fairbairn, 1967), while linoleic acid was shown to be deposited in lipid droplets within the medullary parenchyma of the same species (Lumsden and Harrington, 1966). More recently *H. diminuta* has been shown to absorb oleic and linoleic acids and monoolein when these fats were in the presence of sodium taurocholate (Bailey and Fairbairn, 1968).

There is evidence that *H. diminuta* is incapable of *de novo* synthesis of higher fatty acids and therefore must rely on exogenous sources, although the tapeworm can carry out chain lengthening of absorbed fatty acids (Jacobsen and Fairbairn, 1967).

Arme and Read (1968) have demonstrated that there is a membrane locus involved in the mediated transport of short chain fatty acids by *H. diminuta*. It was therefore thought to be of interest to investigate the uptake of higher fatty acids with particular reference to the initial velocity of this transport.

### MATERIALS AND METHODS

The methods used in the present study for the maintenance of *Hymenolepis diminuta* in the laboratory and the study of membrane transport *in vitro* were essentially those described by Read, Rothman, and Simmons (1963). The outline of these methods is as follows: Young male rats (Holtzman Rat Co., Madison, Wisconsin) were infected *per os* with 32 cysticercoids of *H. diminuta*, obtained

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from infected *Tenebrio molitor*. Rats received a diet of Purina Laboratory Chow. At 10 days post-infection rats were killed and the intestines removed to Krebs-Ringer-tris solution (KRT) at pH 7.4. Worms were flushed from the intestines with KRT and washed several times; they were then randomized into groups of five. Each group of five worms was preincubated in 10 ml of KRT for 30 minutes at 37° C and was then transferred to 4 ml of incubation medium for 2 minutes. Following incubation, worms were washed twice in KRT, dried on Whatman No. 5 paper and transferred to 2 ml of 70% ethanol. Worm lipid was extracted in ethanol for 24 hrs, after which worms were removed to a drying oven at 90° C. Worm dry weight was recorded after drying for 24 hours. Aliquots (1 ml) of ethanol extracts were pipetted on aluminum planchets (1.25 in diameter) and 0.25 ml of a 10 mM sodium bicarbonate solution added. Radioactivity was determined with a Nuclear Chicago gas flow counter.

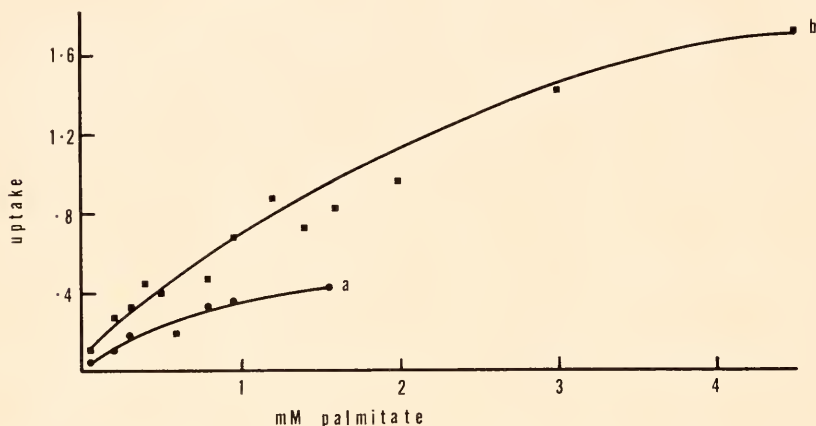


FIGURE 1. The uptake of  $^{14}\text{C}$ -palmitate as a function of palmitate concentration. Curve a is uptake of palmitate diluted from a 2 mM stock solution and Curve b is uptake of palmitate diluted from a 10 mM stock solution. Each point is the mean of at least 8 replicates. Uptake is expressed in  $\mu\text{Moles/g/2 min}$ .

Unless otherwise stated, all incubation media contained sodium desoxycholate ( $1\mu\text{Mole/ml}$ ).

Radioactive palmitic acid, labeled with  $^{14}\text{C}$  at the carbon-1 position, was obtained from Nuclear Chicago Corps. The specific activity was  $38.5\mu\text{C/mg}$ . Unlabeled sodium palmitate (A grade) was obtained from K & K Laboratories. "A" grade stearic, oleic, linoleic, and linolenic acids, and sodium taurocholate were obtained from Calbiochem. Chromatographically pure pentadecanoic, arachidic, and lignoceric acids were obtained from Sigma Chemical Co. and sodium desoxycholate, of enzyme grade purity, was purchased from Mann Research Laboratories. All other chemicals used were of reagent grade. The sodium salts of fatty acids were prepared by titration to pH 7.6–8.0 with sodium hydroxide.

Stock solutions of  $^{14}\text{C}$ -palmitate (100 ml) were made up to contain either  $0.5\mu\text{C}/\mu\text{Mole}(2\text{mM})$  or  $0.1\mu\text{C}/\mu\text{Mole}(10\text{mM})$ . These solutions were diluted to

the required final concentration with 2X KRT, bile salt, water, and other additions where required.

It is necessary to point out that the physical properties, particularly viscosity, of the incubation media varied according to the total concentration of fatty acid salt(s) present. For example, a medium that contained 0.025  $\mu$ Moles of  $^{14}\text{C}$ -palmitate/ml (the lowest concentration used) readily transmitted light whereas a solution that contained 4.5  $\mu$ Moles of  $^{14}\text{C}$ -palmitate/ml was completely opaque. Furthermore, when an additional fatty acid was included in the medium, the concentration of palmitate plus additional fatty acid required to produce definite opacity was lower than that of palmitate alone. No incubation medium was used that contained a flocculent precipitate. It is evident that the solubility of higher fatty acids is such that it imposes limitations on the concentration range over which the transport of long-chain fatty acids may be examined.

In the following account all uptake data are expressed in terms of  $\mu$ Moles  $^{14}\text{C}$ -palmitate absorbed/g dry weight/2 min incubation.

## RESULTS

Unless otherwise stated, the final concentration of  $^{14}\text{C}$ -palmitate in the incubation media was achieved by dilution of a 2 mM stock solution.

Initial studies were carried out to determine the amount of labeled palmitate absorbed by *H. diminuta* during 2 min incubations. Figure 1 (curve a) shows that, over a concentration range of 0.025–1.55 mM palmitate, uptake was non-linear and approached saturation. From these data it was possible, by plotting the reciprocal of uptake against the reciprocal of palmitate concentration (Lineweaver and Burk, 1934), to determine the transport constant,  $K_t$  (equivalent to Michaelis' constant,  $K_m$ ), and the  $V_{\max}$  (extrapolated maximum velocity of a saturable system).  $K_t$  was calculated to be 0.71 mM and  $V_{\max}$  0.48  $\mu$ Moles/g/2 min.

### *Effects of fatty acids on palmitate transport*

A wide variety of fatty acids were investigated for their effects on palmitate uptake by the tapeworm. In initial experiments palmitate concentration was kept constant (0.025 mM) while the concentration of the additional fatty acid was increased from parity with palmitate to its upper limiting physical concentration (i.e., the concentration at which precipitation occurred). The results of experiments using long chain fatty acids are shown in Figure 2. Effects on palmitate uptake were separable into three categories: Group I (Fig. 2a) stimulated uptake, the degree of stimulation being proportional to the concentration of additional fatty acid. Only laurate (12:0) produced an effect of this type. Group II (Fig. 2a, b) comprised those saturated fatty acids [myristate (14:0), pentadecanoate (15:0), heptadecanoate (17:0), stearate (18:0), arachidate (20:0), and lignocerate (24:0)] that inhibited palmitate uptake over their entire concentration range. In no case was the uptake of palmitate fully inhibited; the maximum per cent inhibition varied with the fatty acid present. Group III (Fig. 2c) contained unsaturated fatty acids [oleate (18:1), linoleate (18:2), and linolenate (18:3)]. At concentrations approximately equal to the concentration of palmitate, these fatty

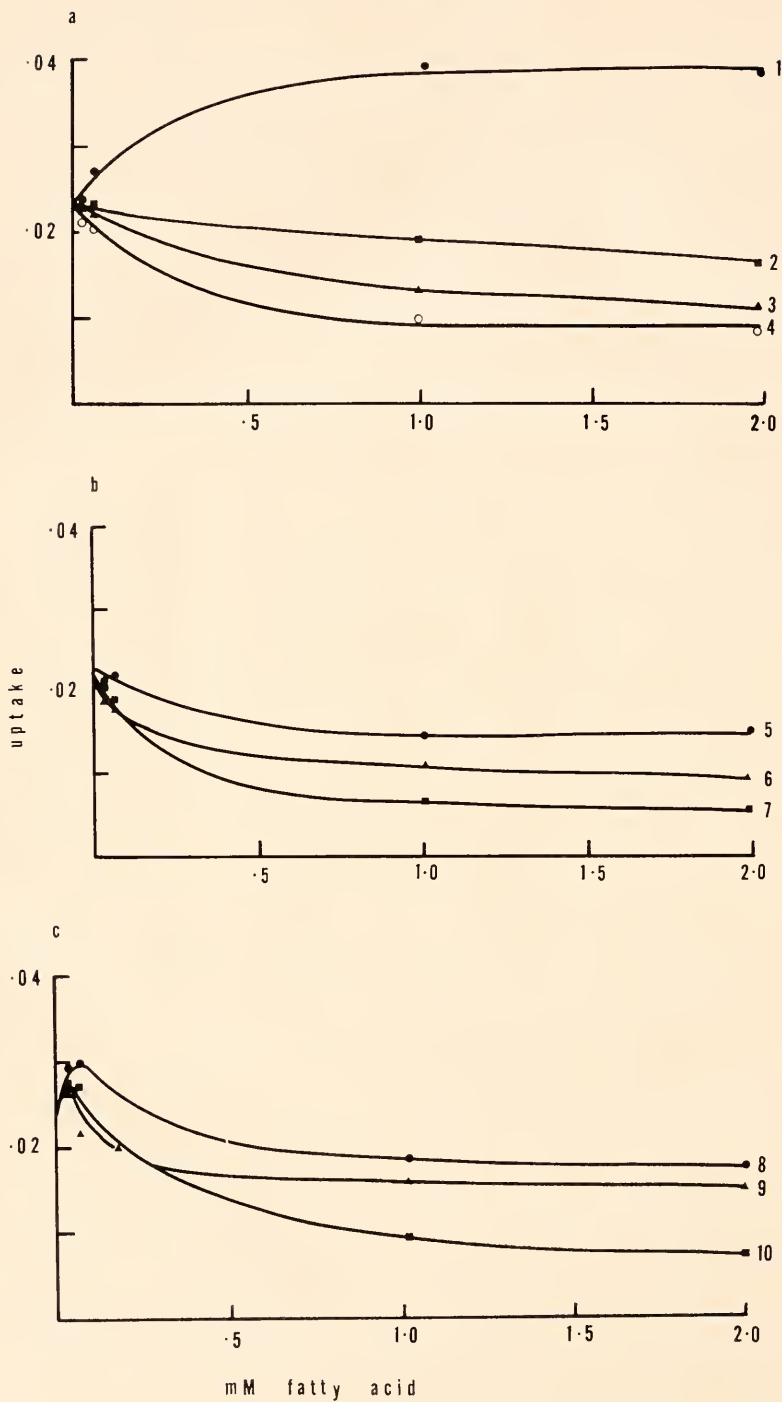


TABLE I  
Effects of sodium salts of fatty acids on  $^{14}\text{C}$ -Palmitate uptake

Palmitate concentration	Addition	Ratio addition: Palmitate	Uptake $\pm$ standard error $\mu\text{Moles g/2 min.}$	$c_f$ Effect	N	P	$K_i$	Effect
0.1 mM	None	—	$0.074 \pm 0.005$	—	16	—	—	—
0.1 mM	Formate	40:1	$0.070 \pm 0.004$	5.4	8	$>0.6$	—	None
0.1 mM	Acetate	40:1	$0.069 \pm 0.004$	6.3	8	$>0.5$	—	None
0.1 mM	Propionate	40:1	$0.074 \pm 0.004$	0.7	8	$>0.9$	—	None
0.1 mM	Butyrate	40:1	$0.078 \pm 0.005$	6.0	8	$>0.6$	—	None
0.025 mM	None	—	$0.022 \pm 0.002$	—	80	—	—	—
0.025 mM	Decanoate	40:1	$0.024 \pm 0.000$	8.2	8	$>0.1$	—	None
0.025 mM	Laurate	40:1	$0.039 \pm 0.002$	75.6	8	$<0.001$	—	Stimulation†
0.025 mM	Myristate	40:1	$0.019 \pm 0.001$	15.5	8	$<0.01$	—	Inhibition†
0.025 mM	Pentadecanoate	40:1	$0.013 \pm 0.000$	41.6	8	$<0.001$	4.9	Inhibition†
0.025 mM	Heptadecanoate	40:1	$0.009 \pm 0.000$	57.5	8	$<0.01$	2.3	Inhibition†
0.025 mM	Stearate	40:1	$0.011 \pm 0.000$	49.1	8	$<0.01$	2.3	Inhibition†
0.025 mM	Arachidate	40:1	$0.014 \pm 0.000$	37.2	8	$<0.001$	3.5	Inhibition†
0.025 mM	Lignocerate	40:1	$0.007 \pm 0.000$	67.3	8	$<0.001$	—	Inhibition†
0.025 mM	Oleate	1:1	$0.029 \pm 0.001$	31.4	8	$<0.001$	4.5	Stimulation†
0.025 mM	Oleate	40:1	$0.018 \pm 0.000$	17.2	8	$<0.01$	4.5	Inhibition†
0.025 mM	Linoleate	1:1	$0.028 \pm 0.002$	25.2	8	$<0.01$	3.8	Stimulation†
0.025 mM	Linoleate	40:1	$0.015 \pm 0.002$	33.2	8	$<0.001$	3.8	Inhibition†
0.025 mM	Linolenate	1:1	$0.028 \pm 0.001$	27.9	8	$<0.001$	5.6	Stimulation†
0.025 mM	Linolenate	40:1	$0.008 \pm 0.000$	62.8	8	$<0.001$	5.6	Inhibition†
0.025 mM	Palmitoleate	1:1	$0.022 \pm 0.001$	1.3	8	$>0.8$	—	None

† Considered to be significant effects by application of Student's  $t$  test.

acids stimulated the uptake of palmitate. Further increase of fatty acid in the medium brought about inhibition of palmitate uptake.

Table I shows the uptake of palmitate (0.025 mM) in the presence of a number of fatty acids (1.0 mM); also included are the uptake data when unsaturated fatty acids were present at 0.025 mM. These data indicate the specificity of the palmitate transport site. Short chain fatty acids, formate through decanoate, had no effect on palmitate uptake, laurate had a pronounced stimulatory effect, while higher fatty acids showed varying degrees of inhibitory (and, in three cases, stimulatory) effect. The only higher fatty acid which was without effect on palmitate transport was palmitoleate ( $\text{CH}_3(\text{CH}_2)_5\text{CH}:\text{CH}_2)_7-\text{COOH}$ ).

was more meaningful, however, to use a  $K_t$  and  $V_{\text{max}}$  determined within each

To determine the nature of the inhibition of palmitate uptake by higher fatty acids, experiments were carried out in which the inhibitor concentration was maintained at 1.5 mM (a concentration above which no further inhibition occurred) and palmitate concentration was increased from 0.025 mM to 0.3 mM. Analysis of these data by the method of Lineweaver and Burk (1934) indicated that inhibitions by Group II and III fatty acids were competitive in nature. Because of

FIGURE 2. The effects of increasing concentrations of various fatty acids on the uptake of palmitate (0.025 mM). 1: laurate, 2: myristate, 3: pentadecanoate, 4: heptadecanoate, 5: arachidate, 6: stearate, 7: lignocerate, 8: oleate, 9: linoleate, 10: linolenate. Each point is the mean of 8 replicates.



their apparent insolubility, inhibitions with myristate and lignocerate could not be analyzed in this way. Using the previously determined  $K_i$  and  $V_{max}$  for the uninhibited uptake of palmitate, it was possible to calculate the inhibitor constants for these fatty acids applying the equation given by Arme and Read (1968); it experimental group. The  $K_i$  for each inhibitory fatty acid is given in Table I. It will be noted that, while linolenate inhibited palmitate uptake by more than 60% its  $K_i$  was 5.6, an unexpectedly high value. (The smaller the  $K_i$ , the greater the inhibitor affinity for the transport site and hence the greater the per cent inhibition.) An explanation for this apparent discrepancy will become evident when the diffusion component of palmitate uptake is discussed below. Inhibitor constant,  $K_i$ , could not be determined for lignocerate due to the formation of a precipitate at higher concentrations. However, by applying the method of Dixon (1953), all inhibitory fatty acids, with the exception of oleate, linoleate, and linolenate, were shown to be partially competitive inhibitors of palmitate transport.

#### *Effects of compounds other than fatty acids on palmitate uptake*

To ascertain the degree of specificity of the palmitate transport site, a variety of biologically important substances were examined for their effects on palmitate transport. In these experiments palmitate concentration was maintained at 0.1 mM while the test substances were kept at a concentration of 4.0 mM. None of the following inhibited palmitate uptake: succinate, malate, glutamate, aspartate, citrate, adenine, uracil, betaine, ouabain, dextrose, galactose, leucine, alanine, phenylalanine, sarcosine, lysine and 2,4-dinitrophenol (at 2.5 mM).

#### *A diffusion component in palmitate uptake*

The uninhibited uptake of palmitate (Fig. 1a) showed non-linearity over the concentration range tested, and it was therefore impossible to determine the significance of diffusion of palmitate through the tegument in this system. However, by reference to Figure 2a, b, and c, it is evident that no inhibitor completely precluded uptake of palmitate. It is, therefore, apparent that there are at least two systems for the entry of palmitate; the first is a transport that can be inhibited to a varying extent by fatty acids, but is unaffected by other groups of compounds, and a second, which is not affected by fatty acids, and may be diffusion. It was possible to investigate this second system further in the following manner: Using heptadecanoate, lignocerate, and linolenate at concentrations above which no further inhibition of palmitate uptake occurred [but varying the absolute concentrations of both palmitate (0.0125 mM, 0.025 mM, and 0.05 mM) and inhibitor (0.25 mM, 0.5 mM, and 1.0 mM) whilst maintaining a constant 20:1 inhibitor to substrate ratio] it was possible to show that palmitate uptake, after inhibition, was linear with respect to its concentration. This relationship indicates first, that residual palmitate uptake following inhibition was diffusion, although it is also possible that the data from these experiments suggest the presence of a second mediated uptake system, saturable at concentrations in excess of those used; and second, that the high per cent inhibitions by lignocerate and linolenate were not competitive beyond the maximum per cent inhibition with heptadecanoate (57.5%). At high concentrations both lignocerate and linolenate produced very viscous media and it seems likely that non-specific interference with palmitate uptake

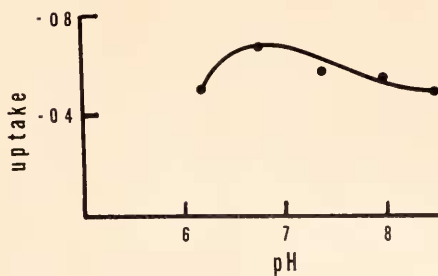


FIGURE 3. The uptake of  $^{14}\text{C}$ -palmitate (0.05 mM) at various pH values. Each point is the mean of 8 replicates.

occurred at such inhibitor concentrations. This would explain the high  $K_i$  value for linolenate and the fact that it was not possible to carry out a Lineweaver-Burk plot with lignocerate. In other words, inhibition of palmitate uptake can occur in one of two ways, either by an inhibitor that interacts with the transport carrier site over its entire concentration range, or, by an inhibitor that, at high concentrations at least, alters the physical properties of the incubation medium and thus interferes with palmitate availability at the carrier site.

#### *Effects of pH on palmitate uptake*

Examination of palmitate uptake from incubation media buffered to a range of pH values with tris-maleic acid buffer (Fig. 3) indicated the occurrence of a pH optimum value, 6.8. The optimum pH appeared to be independent of palmitate concentration (Fig. 4).

#### *Effects of bile salts on palmitate uptake*

Sodium desoxycholate was included in incubation media at a concentration of 1  $\mu\text{Mole/ml}$ . Substitution of taurocholate for desoxycholate had no effect on palmitate uptake; this was examined over a wide range of exogenous palmitate concentrations.

Experiments were carried out to determine a possible role of bile salts in the transport of palmitate. In the complete absence of desoxycholate, palmitate uptake decreased 9.2% to 52.7%, depending upon palmitate concentration in the medium (Table II, column 1). The effects of increasing desoxycholate concentration on

TABLE II  
*Per cent inhibition of  $^{14}\text{C}$ -Palmitate uptake in the  
absence of sodium desoxycholate*

Concentration of Palmitate	Per cent inhibition using diluted 2 mM Palmitate stock	Per cent inhibition using diluted 10 mM Palmitate stock
0.025 mM	9.2	19.1
0.100 mM	26.4	23.1
0.200 mM	52.7	26.5
0.800 mM	30.0	18.4

palmitate uptake are shown in Figure 5. Varying the concentrations from 0.025 mM to 2.0 mM desoxycholate did not alter palmitate uptake, but concentrations of bile salt in excess of 2.0 mM brought about a twofold increase in the transport of palmitate. The effects of increasing the concentration of desoxycholate above 3.0 mM could not be examined due to the formation of an insoluble precipitate.

#### *The use of a 10 mM palmitate stock solution*

In order to extend the range of concentrations over which the uptake of  $^{14}\text{C}$ -palmitate could be investigated, a 10 mM palmitate stock solution was made up (previous data refer to dilutions of a 2 mM palmitate stock). The uninhibited uptake of palmitate diluted from the stronger stock solution is shown in Figure 1, curve b. It is evident that, at all concentrations, the palmitate uptake from a diluted 10 mM stock is greater than that from a diluted 2 mM stock (curve a). The 10 mM curve also showed non-linearity and, hence, a  $K_t$  (1.0 mM) and  $V_{\text{max}}$  (1.4  $\mu\text{Moles/g/2 min}$ ) could be determined.

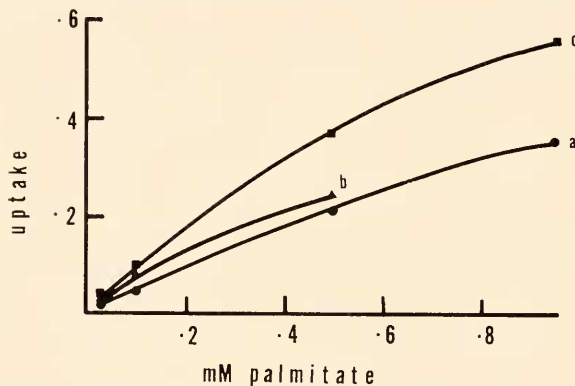


FIGURE 4. The uptake of  $^{14}\text{C}$ -palmitate at pH 6.2 (Curve b), pH 6.8 (Curve c) and pH 8.5 (Curve a) as a function of palmitate concentration. Each point is the mean of 8 replicates.

Because of the differences demonstrated between uptake from dilutions of stocks of different strengths, the effects of various higher fatty acids were investigated using diluted 10 mM stock palmitate. The results of these experiments are shown in Table III. At concentrations of 1.0 mM, all fatty acids investigated, with the exception of heptadecanoate, were more effective inhibitors of palmitate uptake when dilutions from 10 mM stock solutions were used than was the case with dilutions from 2 mM stock solutions. Furthermore, oleate, at a concentration previously shown to be stimulatory (*i.e.*, at the same concentration as palmitate) failed to stimulate palmitate uptake when the 10 mM dilution was used. The effects of depletion of the incubation medium of desoxycholate on palmitate uptake were investigated using 10 mM dilutions. The results of this experiment are shown in Table II, column 2; these results are expressed as per cent reduction in palmitate uptake as a result of omitting the bile salt. At 0.025 mM palmitate the absence of bile induced a greater reduction of palmitate uptake with the 10 mM dilution than



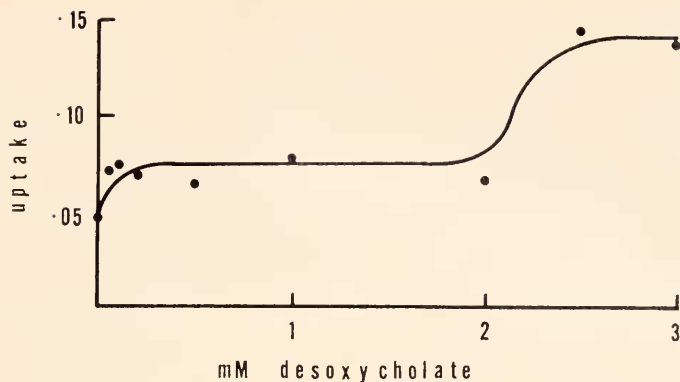


FIGURE 5. The effects on the uptake of  $^{14}\text{C}$ -palmitate (0.05 mM) of increasing concentrations of sodium desoxycholate. Each point is the mean of 8 replicates.

with the 2 mM dilution. At all other palmitate concentrations, this effect was reversed. With both dilutions, the maximum reduction of uptake due to the absence of desoxycholate occurred at 0.2 mM palmitate.

Attempts were made to demonstrate differences in the physical properties of palmitate solutions diluted from 2 mM and 10 mM stock solutions. No osmotic pressure differences, using depression of freezing point method, could be shown. Ultra-centrifugation did not reveal any differences in physical properties and the use of fat-soluble dyes proved to be uninformative.

### DISCUSSION

Arme and Read, (1968) have reviewed the literature concerning the entry of fatty acids into animal tissues. These workers have presented evidence for the mediation of transport of acetate and other short chain fatty acids through the tegument of the tapeworm *Hymenolepis diminuta*. The tegumentary site at which mediation occurred was shown to be specific for saturated fatty acid salts containing less than 9 carbon atoms in the hydrocarbon chain. The present investigation has provided evidence for the existence of a second fatty acid transport system in

TABLE III

*Effect of various fatty acids on the uptake of  $^{14}\text{C}$ -Palmitate (0.025 mM) diluted from a 10 mM stock*

Addition	Ratio addition to Palmitate	Uptake $\pm$ Standard error	N	P	% Effect	Effect
None	—	0.044 $\pm$ 0.001	8	—	—	—
Decanoate	40:1	0.029 $\pm$ 0.001	8	<0.001	34.1	Inhibition†
Heptadecanoate	40:1	0.022 $\pm$ 0.001	8	<0.01	49.0	Inhibition†
Oleate	1:1	0.046 $\pm$ 0.003	4	>0.8	3.1	None
Linoleate	40:1	0.023 $\pm$ 0.002	4	<0.001	46.8	Inhibition†

† Considered to be significant by application of Student's t test.

*H. diminuta*. This system is concerned with the transport of higher fatty acids, and seems to be specific for saturated and unsaturated long chain fatty acids.

Although only  $^{14}\text{C}$ -palmitate has been used as a substrate for the investigation of long chain fatty acid transport, it is not unreasonable to postulate that the various higher fatty acids, shown to be competitive inhibitors of palmitate transport, are themselves transported via the same carrier site. The affinities of these inhibitory acids for the palmitate site are reflected by their inhibitory efficacy and their inhibitor constants. It would appear that those acids most closely resembling palmitate, in terms of chain length, have the greatest affinities for the palmitate transport site. The high per cent inhibitions of palmitate uptake by lignocerate and linolenate are thought to be due, in part, to their physical effects on aqueous solutions of palmitate, particularly when the former acids were present at high concentrations, rather than simply attributable to their interaction with the carrier site. In this way the palmitate site is similar to the acetate-butyrate site in that the site affinity shown by a fatty acid inhibitor is related to the difference between the number of carbon atoms in the substrate molecule and the number of carbon atoms in the inhibitor molecule.

The transport of palmitate by *H. diminuta* resembles the transport of short chain fatty acids (Arme and Read 1968) and of purines and pyrimidines (MacInnis, Fisher and Read, 1965) in that two component pathways were detectable. At low concentrations of palmitate, up to 4.5 mM, diffusion through the tegument appears to be lesser than mediated transport, as shown by saturation kinetics. On the other hand, experiments carried out using inhibitors at concentrations far in excess of palmitate concentration have shown that inhibition never interrupted more than 70% of palmitate uptake. Furthermore, the remaining 30% uptake (in fact, the remaining 42.5% palmitate uptake subsequent to inhibition with heptadecanoate) was found to be linear with respect to palmitate concentration, and therefore thought to be diffusion. It would seem that contradictory data exist; the saturation data indicate that at 0.25 mM little palmitate enters by diffusion, whereas inhibition data indicate that, at the same concentration, over 40% of palmitate uptake is via a diffusion system. This discrepancy might be resolved if it is postulated that the inhibitor, in this case heptadecanoate, interacted with palmitate at the carrier site, thus precluding mediated transport, but had no effect on the diffusion of palmitate, the latter being masked by mediated transport in the absence of inhibitor.

It is of interest to compare the rate of entry of acetate and palmitate through the tegument of *H. diminuta*. Arme and Read (1968) showed the uninhibited uptake of acetate to be  $0.105 \mu\text{Moles/g/min}$  from a 0.1 mM solution; a rate of  $0.210 \mu\text{Moles/g/2 min}$  is derived by multiplication. Under identical conditions, the uninhibited uptake of palmitate was measured as  $0.074 \mu\text{Moles/g/2 min}$ . Jacobsen and Fairbairn (1967) demonstrated that the label from  $^{14}\text{C}$ -acetate was incorporated into *H. diminuta* lipids at a lower rate than that of higher fatty acids, including  $^{14}\text{C}$ -palmitate. Arme and Read suggested that the relatively low incorporation rate of acetate might be due to dilution with endogenously-produced acetate. This would seem to be highly probable in the light of the present demonstration that during two minutes, palmitate is transported at a rate about one-third that of acetate. It is worthy of note to record that Winterbourn and Batt (1968) observed a "high incorporation" rate of plasma  $^{14}\text{C}$ -palmitate into the lipids of

bovine leukocytes. It would be of interest to know the relationship between the rates of incorporation and uptake in the latter system and also to compare these with rates of incorporation and uptake of acetate.

A number of workers investigating membrane transport have observed that apparent synergism may occur when two chemically similar substances are included in a single incubation medium. Schafer and Jacquez (1967) observed that the uptake of L-tryptophan was stimulated in the presence of equimolar concentrations of either L-leucine, DL-p-fluorophenylalanine or L-methionine. These authors considered these synergistic phenomena to be "competitive stimulations." Previously, Jacquez (1961, 1963) recorded that while a number of neutral L-amino acids would stimulate the uptake of L-tryptophan by Ehrlich ascites tumour cells, when the concentrations of both substrate and stimulator were equal, an increase in the concentration of the stimulatory amino acid induced inhibition of tryptophan uptake. Certain observations made in the present study are remarkably similar to those of Jacquez (1961, 1963). Oleate, linoleate, and linolenate, when present at the same concentration as palmitate, stimulated the uptake of palmitate by 31.4%, 25.2%, and 27.9%, respectively. When the concentrations of any one of these unsaturated acids was increased, inhibition of palmitate uptake occurred. Only one unsaturated fatty acid tested, palmitoleic, failed to stimulate palmitate entry at a 1:1 ratio.

It is considered that the above stimulations of palmitate uptake cannot be termed competitive stimulations since it has been shown, by application of the methods of both Dixon, and Lineweaver and Burk, that these three unsaturated fatty acids are, in fact, competitive inhibitors of palmitate transport. On the other hand, it was demonstrated that laurate would stimulate palmitate transport irrespective of its concentration in the medium and that, furthermore, stimulation was related to laurate concentration. Again, by application of Dixon's method, laurate was shown to be a partially competitive stimulator of palmitate uptake. MacInnis *et al.* (1965) observed that thymine competitively stimulated the transport of uracil by *H. diminuta*. They considered that this phenomenon might be accounted for either by the prevention of uracil efflux by thymine or by the combined effect of one molecule of uracil and one molecule of thymine at the carrier site. They further pointed out that their data did not exclude possibility of dimerisation as a prerequisite of membrane transport.

The two types of stimulation discerned in the uptake of palmitate do not seem to be related. Laurate is known to increase the solubility of fatty acids in organic solvents (Fieser and Fieser, 1959), a property apparently unique to this fatty acid. Its stimulatory effect on palmitate transport may, therefore, reflect its effect on the solubility of palmitate rather than a direct effect on the carrier site. No explanation can, at present, be offered to account for the stimulation of palmitate uptake by three unsaturated fatty acids, especially since palmitoleate failed to be stimulatory. All four unsaturated fatty acids investigated occur in the *Cis* form (Heilbron, Cook, Bunbury and Hey, 1965) thus ruling out certain stereo-chemical effects.

The effects of bile salts on palmitate transport have been examined in the present study. Sodium desoxycholate is known to form specific intermolecular complexes with fatty acids; such compounds are termed choleic acids (Fieser and Fieser 1959). The formation of a bile-fatty acid complex (not to be confused

with a micelle) markedly increases the solubility of the fatty acid and it was thought that this was possibly the way in which desoxycholate could increase the uptake of palmitate. However, evidence has been accumulated which suggests that, under the experimental conditions described, choleic acids are not formed, or if they are, they exert no effect on palmitate transport. Apparently taurocholate does not form intermolecular inclusion complexes (Fieser and Fieser 1959), and it was found that taurocholate could replace desoxycholate without altering palmitate uptake. Choleic acids are composed of a specific number of molecules of desoxycholate enclosing a single molecule of fatty acid. In the case of palmitate, eight molecules of desoxycholate are required for each palmitate molecule. Therefore, if choleic acid formation is a prerequisite of optimum uptake, the initial ratio of desoxycholate to palmitate in the medium will be critical. However, this has not been found to be so (Fig. 5). The twofold increase in palmitate entry recorded when the concentration of desoxycholate exceeded 2.0 mM may be accounted for in one of two ways. First, the tegument of the worm may be rendered more permeable to palmitate due to the direct effect of relatively high levels of bile salt, or second, at such concentrations, the bile salt may be existing in predominantly micellar form (Bailey and Fairbairn 1968). Above its Critical Micelle Concentration, a surfactant, such as bile salt, will have distinct effects on aqueous solutions of fatty acids, particularly in the direction of solubilization. In contrast to palmitate, the uptake of  $^{14}\text{C}$ -acetate was unaffected by desoxycholate, irrespective of the concentration of the latter. This may be taken as evidence that bile salt exerts its influence at higher concentrations in micellar form.

In the mammalian jejunum, bile salt (taurodesoxycholate) is absorbed at a rate similar to oleate (Gordon and Kern, 1968) indicating the existence, and importance to fatty acid transport, of bile salt-lipid micelles. Nonetheless, with the exception of the experiments employing unusually high levels of bile salt, there is no evidence that micelles exist in the present incubation media. Application of the expression:

$$\log \text{Critical Micelle Concentration} = A - B.nC,$$

[where A and B are physical constants for homologous series of fatty acid salts and nC is the number of carbon atoms in the hydrocarbon chain (Osipow, 1962)], has indicated that, under the experimental conditions used, the CMC for palmitate is 18.7 mM.

The observation that palmitate uptake from a solution of given strength varies according to the strength of the stock solution from which it was diluted, has proved difficult to interpret. It is unlikely that a 0.025 mM palmitate solution made by diluting a 2 mM stock is qualitatively different from a 0.025 mM solution made by diluting a 10 mM stock; it is reasonable to suppose that these two solutions differ only in the proportions of a particular moiety. It was originally conjectured that the 10 mM stock solution of palmitate contained a greater number of micelles than did its weaker counterpart, and that micellar fatty acid was transported more efficiently than the ionic form. However, it has become evident that neither of the stock palmitate solutions contained micelles, at least in significant number. At concentrations below their critical micelle concentration aqueous solutions of amphipaths contain free ions in equilibrium with small aggregates (Osipow, 1962). Little is apparently known about the size or preponderance of these aggregates at various



amphipath concentrations. It is, therefore, postulated that the 10 mM stock palmitate solution contains either a greater number of molecular aggregates or that these aggregates are of larger size than are found in the 2 mM solution. Dilution of these stock solutions apparently does not alter the nature of the aggregates. Thus, while both 2 mM dilutions and 10 mM dilutions, at the same final concentration of palmitate, are ostensibly identical, they possess physical dissimilarities that are manifested in differing rates of palmitate transport. In no way has it been possible to demonstrate the actual nature of these differences between the two palmitate solutions. In all cases but one, the effects of other fatty acids on the transport of palmitate diluted from a 10 mM stock have been to increase inhibition of uptake or lower stimulation, depending upon the particular additional fatty acid. All solutions of fatty acids, other than labeled palmitate, were made up to contain 5  $\mu$ Moles/ml. It is probable that these solutions also contain ions and aggregates, and that they will differ, in terms of number or size of these aggregates, from both the 2 mM and 10 mM diluted solutions when they, themselves, are diluted. Hence, if aggregated molecules of palmitate are more rapidly transported, it might be expected that aggregated molecules of other fatty acids might act more efficiently as inhibitors than solutions containing less or smaller aggregates.

A further piece of evidence for the relationship between fatty acid transport and the presence of molecular aggregates lies in the quantitatively different effects on palmitate uptake when diluted 2 mM and 10 mM stocks are used in the absence of bile salt. At all but the lowest palmitate concentrations (Table II) the effect of removing bile salt from the incubation media was less pronounced when the medium contained a 10 mM dilution. If it is postulated that bile salt, even at concentrations below its critical micelle concentration, functions as an aggregator of fatty acid, then the presence of preformed aggregates of palmitate might be expected to diminish the effects of depleting and removing bile salt from the incubation media.

It is thought that aggregates of palmitate are unlikely to be transported in their entirety, but serve merely to bring a greater number of fatty acid molecules within the proximity of the membrane carrier sites than would occur if ions existed independently. That is to say that, when a single molecule, as part of a poly-molecular aggregate, attaches to the carrier site, it predisposes other molecules of the aggregate for transport.

The effects of pH on palmitate uptake are reminiscent of the pH effect on an enzymic reaction, in that a pH optimum was detected. It has been calculated that sodium palmitate is more than 90% ionized in the pH range of this investigation and consequently the observed pH optimum is somewhat difficult to interpret.

The present investigation has shown that the tapeworm *H. diminuta* possesses a fifth type of mediated transport system situated on or in the tegument. The higher fatty acid transport site resembles other transport systems in its degree of specificity, whereas the differences that have been demonstrated are thought to represent the physical properties of aqueous solutions of fatty acids and bile salts rather than intrinsic differences in the carrier site.

#### SUMMARY

1. The uptake of  $^{14}\text{C}$ -palmitate by *Hymenolepis diminuta* has been shown to occur via a mediated process at concentrations of up to 4.5 mM palmitate. Entry



of palmitate by diffusion is not thought to be significant under present conditions.

2. Fatty acids containing less than 12 carbon atoms and substances other than fatty acids did not inhibit palmitate uptake. Laurate, at all concentrations, stimulated uptake, while saturated fatty acids with up to 24 carbon atoms inhibited uptake to varying degrees. Three of the four unsaturated fatty acids tested stimulated palmitate uptake when present at concentrations equal to that of palmitate and inhibited uptake when their concentrations were increased. All saturated fatty acids that affected palmitate uptake were found to be partially competitive inhibitors or stimulators.

3. A pH optimum was detected for palmitate uptake.

4. The effects of bile salts on palmitate uptake were investigated, but the role of bile in the transport of palmitate could not be elucidated.

5. The differences in palmitate uptake following dilution of 2 mM and 10 mM palmitate stock solutions are thought to reflect the physical properties of fatty acid solutions. It is postulated that 10 mM dilutions of palmitate contained either a greater number of, or larger, aggregated molecules than were present in 2 mM dilutions. It is considered that enhancement of palmitate uptake is related to the presence of these molecular aggregates.

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